Review
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Unknown Aspects of Well-known Cardiovascular Diseases: Identification of Novel miRNA Genes

İyi Bilinen Kardiyovasküler Hastalıkların Bilinmeyen Yönleri; Yeni Tanımlanmış miRNA Genleri

miRNA Genes of the Cardiovascular Diseases

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Özet

Kodlanmayan küçük ribonükleik asit (miRNA)'lerin bulunması ve işlevlerinin tanımlanmasıyla, RNA'ların canlı yaşamı için çok önemli süreçlerde rol oynadıkları belirlenmiştir. miRNA'larda meydana gelen bozukluklar birçok hastalığa yol açmaktadır. Beklendiği üzere kanserler, nörodejeneratif hastalıklar ve immün yetmezlik gibi hastalıklarla ilişkilendirilmiş ve son yıllarda yeni yapılan çalışmalarla kardiyovasküler hastalıklarla olan ilişkilileri gösterilmiştir. Bu miRNA'lar, yeni tedavi yaklaşımlarında hem hedef hem de araç olarak görülmektedirler. İnsan genomundaki tüm miRNA'ların işlevlerinin aydınlatılmasıyla yeni tedavi yaklaşımları geliştirilebilecektir. Bu derlemede, miRNA'ların kardiyovasküler hastalıklarla ilişkili olduğu kuvvetli çalışmalarla ispatlanan çeşitleri, kardiyovasküler hastalıklarla olan ilişkileri, ve tanı-tedavi amaçlı kullanımlarıyla ilgili literatüre dayalı değerlendirme yapılmıştır.

Anahtar Kelimeler

Kardiyovasküler Hastalık; Ateroskleroz; Miyokard Enfarktüsü (MI); Mikro RNA (miRNA)

Abstract

The discovery of noncoding small ribonucleic acids (miRNAs) in recent years and identification of their functions have indicated that RNAs play a very important role for living organisms. Deregulation of miRNAs has been implicated in many diseases such as cancers, neurodegenerative diseases and immunodeficiency, as well as, with the new data emerging, cardiovascular diseases. miRNAs have become targets and tools of novel therapeutic approaches. By identifying all functional miRNAs that are encoded in the human genome, new therapeutic approaches may be developed and clinical trials using miRNA-based molecules may be achieved. In this paper, we review the literature regarding the miRNAs that have been associated with cardiovascular disease and new diagnostic/therapeutic approaches.

Keywords

Cardiovascular Disease; Atherosclerosis; Myocardial Infarction (MI); MicroRNA (miRNA)

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Introduction

Cardiovascular diseases (CVD) are quite prevalent with high morbidity and mortality levels. In recent years, despite increased awareness of CVD and the associated risk factors, such diseases are still considered to be the leading cause of death in both developed and developing countries [1]. Given the data made available in Europe, CVDs are found to cause more than 4.35 million deaths on average per year, and they are determined to be the definitive cause for almost half of all deaths in both males and females [2].

CVD include coronary heart disease, cerebrovascular diseases (stroke), peripheral vascular diseases as well as thoracic and abdominal aortic aneurysm. Thus, CVD stands for a group of diseases that affect the whole vascular bed. Naturally, a local pathology may imply an existing or possible disease that may occur in another part of the vascular tree, since their pathogenesis is similar over the atherosclerotic base on the same vascular bed. Early diagnosis, follow-up and treatment parameters are inadequate for this disease or disease group, and the search for new biological indicators continues.

In 1993, the discovery of small RNA particles (app. 20-25 nucleotides) regulating post-transcriptional gene expression in humans as well as studies examining their functions and significance opened up a wholly new discourse in the scientific community. These particles are called micro RNAs (miRNA) [3].

Since their discovery, many different miRNAs have been identified as biological indicators in the diagnosis and follow-up of many diseases. At the same time, miRNAs are suggested to be drug targets in disease modeling studies conducted to develop new treatment methods specific to diseases [4-7]. miRNAs, identifiable in the circulation particularly in CVD such as myocardial infarction (MI), atherosclerosis, coronary artery disease (CAD), heart failure (HF), atrial fibrillation (AF), cardiac hypertrophy (CH) and cardiac fibrosis (CF), may be used as disease-specific biological markers [8-12].

This study underlines the importance of miRNAs in CVD in terms of diagnosis, follow-up and treatment, and associates relevant miRNAs with the pathophysiology of diseases with respect to the literature available.

Micro RNA

miRNAs are short non-coding RNAs of approximately 22 nucleotides and regulate gene expression post-transcriptionally. They were identified in 1993 for the first time by Lee et al. who studied the development of C. elegans [13,14]. Lin-4 was the first miRNA discovered [13,15–17]. Studies conducted so far have identified 2,588 human pre-miRNA sequences that are recorded in the "miRBase release 20" database. These miRNAs are demonstrated to be most dense on chromosome 1 (161 miRNAs) and least dense in the Y chromosome (2 miRNAs). Researchers found that more than 50% of human miRNAs are located in cancer-related genomic regions and regions near fragile sites [18]. MiRNAs may play roles not only in post-transcriptional regulation of gene expression but also in a variety of biological processes such as development, differentiation, apoptosis, cell proliferation and cancer [5,13,19]. It is suggested that aberrant expression miRNAs cause a broad range of diseases from myocardial infarction to autoimmune diseases.

miR106b is a microRNA that has been reported to alter the expression of the genes responsible for heart failure [20]. In addition, miR-106b is suspected of being associated with schizophrenia as well [21]. miR-143 is reported to be associated with obesity, targeting the ERK5 protein of the Mitogen Activated Protein (MAP) kinase signaling pathway [22]. miRNAs may also indirectly affect gene expression through epigenetic mechanisms. Deregulation of miR-101, caused by the Hepatitis B virus, was reported to affect DNA methylation by targeting DNA methyl transferase 3A [23]. Today, it is estimated that approximately 30-60% of all human genes are regulated by miRNAs [24].

Micro RNA Biogenesis

The biogenesis of miRNAs consists of a series of processes starting from the cell nucleus and ending in the cytoplasm [25]. miRNAs are mostly transcribed by RNA polymerase II in the form of 1 kb long pri-miRNAs. The pri-miRNAs thus generated bear 7 methyl guanosine cap at 5' and polyA tail at their 3' end, similar to mRNAs. The hairpin structure of pri-mRNAs is recognized by Drosha that acts together with DGCR8 (Di George Critical Region 8), an RNase-3 enzyme and cleaves it. This process generates a pre-miRNA of about 70 nucleotides. This structure is then exported through the nuclear pores to the cytoplasm via RAN GTP and Exportin-5, i.e. the nuclear transport complex. Once in the cytoplasm, the pre-miRNA is cut into double-stranded mature miRNAs of approximately 22 nucleotides by Dicer, together with TRBP, another RNase-3 enzyme that can bind to double-stranded RNA. One strand is selected according to its thermodynamic characteristics to bind to the complementary 3'UTR of the target mRNA and degrade the mRNA or inhibit its translation; the Argonaute (Ago) protein and the RISC complex (RNA-Induced Silencing Complex) are also involved in the process (Figure 1) [26].

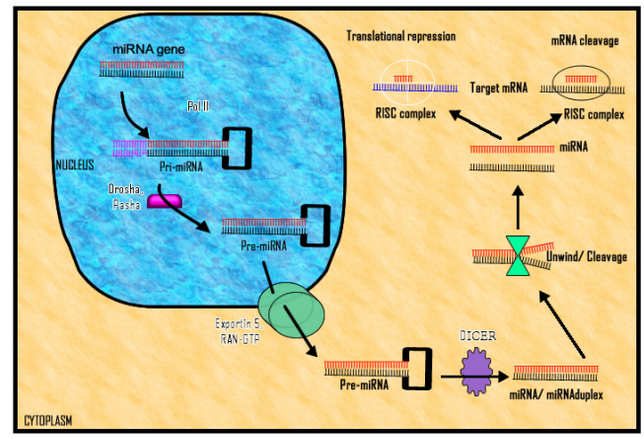

Figure 1. miRNAs' biogenesis and action mechanism

Mature miRNA binds to the target mRNAs via six-nucleotide complementary sequences located between 2nd and 7th nucleotides at the 5' end. This region is called the seed sequence. Studies indicate that the complementary sequence at the seed suffices to suppress the target mRNA. The seed sequence has four binding regions which differ depending on whether it complements the mRNA or not [17]. They are characterized as follows.

- 6mer: Pairing includes six nucleotides at the seed region only,
- 7mer-A1: Nucleotide pairing at the seed, and miRNA's 1st nucleotide corresponding to the Adenine (A) nucleotide,
- 7mer-m8: Pairing of miRNA's 8th nucleotide in addition to the nucleotide pairing at the seed,
- 8mer: Characterized by nucleotide pairing at seed region and

Table 1. The miRNA genes identified for cardiovascular diseases and related pathologic pathways

Disease	miRNA	Related Pathway	Ref.
Atherosclerosis, Myocardial Infarction, Myocardial Reperfusion Injury, Cardiomyocytes metabolism	miR-21	• Found high levels of expression for all of them in quiescent endothelial cells, • Regulates Vascular Smooth Muscle Cell Function via Targeting Tropomyosin 1 in Arteriosclerosis Obliterans of Lower Extremities, • Up-regulated in human atherosclerotic plaques compared to non-atherosclerotic left internal thoracic arteries in the Tampere Vascular Study, • Down-regulated in infarcted areas, up-regulated in border areas, • Protect infarcted area by targeting Programmed cell death protein 4(PDCD-4), • Contributes to myocardial disease by stimulating Mitogen-activated protein (MAP) kinase signaling in fibroblasts, • Downstream effector of Akt signaling pathway that mediates its anti-apoptotic effects via suppression of Fas ligand, • Protects against the H2O2-induced injury on cardiac myocytes via its target gene PDCD4	29,30, 32,44, 48,
Atherosclerosis, Coronary Artery Disease (CAD), MI	miR-126	• found high levels of expression for all of them in quiescent endothelial cells, • Significantly decreased in patients with CAD and high low-density lipoprotein (LDL) cholesterol level. • Inversely correlated with MI risk, • Decreased in patients with acute MI after the onset of symptoms compared with healthy subjects.	29,30, 37, 49
Atherosclerosis	miR-221	• Found high levels of expression for all of them in quiescent endothelial cells	29-32
Atherosclerosis	miR-222	• Found high levels of expression for all of them in quiescent endothelial cells	29-32
MI	miR-223	• Showed positive associations with MI risk	29-32
MI	miR -106b	• Modulates apoptosis and angiogenesis in MI.	20,21,32
MI	miR -92a	• Up-regulated in STEMI (ST-segment elevation MI patients, • Survival rate is higher in patients showing down-regulation of miR-92a.	32, 33
Atherosclerosis	miR -143	• Suppress atherogenesis.	22, 34, 35
Atherosclerosis	miR -145	• Suppress atherogenesis, • Downregulation of miR-145, which controls differentiation of smooth muscle cells, promotes lesion formation	22, 34, 35
Ischemia / Reperfusion Injury	miR-494	• Targets both proapoptotic and antiapoptotic proteins, • Protects against ischemia/reperfusion-induced cardiac injury,	38
Myocardial Ischemia / Reperfusion Injury	miR-15a/b	• Up-regulated in response to myocardial ischemia/reperfusion injury.	40
CAD, MI	miR-1	• Overexpressed in patients with CAD and that overexpression of miR-1 in a rat model of cardiac infarction exacerbated arrhythmogenesis, • Regulates cardiac hypertrophy which is significantly down-regulated in hypertrophic tissue, • Increased level indicates myocardial damage, in patients with cardiovascular disease, • Increased level indicates myocardial damage, in patients with acute MI, • Embryonic stem cells overexpressing microRNA-1 attenuate apoptosis in the injured myocardium.	8, 38, 41,42
MI	miR-206	• Upregulated expression in a rat model,	41
Atherosclerosis, Myocardial Reperfusion Injury	miR-146a	• Induces the protein expression of Tumor necrosis factor (TNF)-α, Monocyte chemoattractant protein-1 (MCP-1), Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappa) p65, which are key pro-inflammatory cytokines and critical transcription factor in atherosclerosis, • Up-regulated in human atherosclerotic plaques (compared to non-atherosclerotic left internal thoracic arteries (LITA) in the Tampere Vascular Study, • Increased expression of microRNA-146a decreases myocardial ischemia/reperfusion injury,	8, 46
MI	miR-150	• Decreases in MI patients with ventricular rupture ,	8
Atherosclerosis, Ischemia, MI	mir-155	• Elevates in proinflammatory macrophages and atherosclerotic lesions, • Inhibition of miR-155 protects against cardiac ischemic Injury, • Decreases in MI patients with ventricular rupture.	8, 52
MI, Cardiomyocytes metabolism,	miR-133	• Increases in patients with myocardial damage, • Increases in patients with MI, • Regulates the expression of Glucose transporter type-4 (GLUT-4) by targeting Krüppel-like factor-15 (KLF-15) and is involved in metabolic control in cardiac myocytes.	8, 42
MI	miR-328	• Increases in patients with MI,	8
Endomyocardial Fibrosis, MI	miR-208a	• Is essential for expression of the genes involved in cardiac fibrosis and hypertrophic growth, • May be a novel biomarker for early detection of myocardial injury in humans plasma, • May be a biomarker of MI,	8, 28, 43
MI	miR-208b	• Increases in patients with MI,	8, 28, 43
MI	miR-499	• May be a novel biomarkers of AMI,	8, 43
MI	mir-30a	• May be a potential indicators for AMI,	10
Atherosclerosis	miR-10a	• Contributes to the regulation of proinflammatory endothelial phenotypes in athero-susceptible regions,	47
MI	miR-24	• Regulates vascularity after MI, • Regulates cardiac fibrosis after MI.	45

MI: Myocardial Infarction , CAD: Coronary Artery Disease

nucleotide 8 of miRNA, and nucleotide A located against nucleotide 1 of miRNA [17, 27].

Micro-RNAs in Cardiovascular Diseases

Many studies have already demonstrated the critical roles played by miRNAs in cardiovascular biology, and recent studies suggest that miRNAs may become new treatment agents in the near future (Table-1). To shed light on this subject, this study will summarize the latest developments by focusing particularly on endothelial pathogenesis and atherogenesis in cardiovascular diseases.

• The relation between endothelial functions and miRNA:

Dicer, a key enzyme for miRNAs' biological process, is active in many pathways across a broad range of functions of endothelial cells [25, 28]. Studies indicate that suppression of Dicer resulted in the dysregulation of miRNAs including let-7, miR-21, miR-126, miR-221 and miR-222, and reduced inflammatory cytokines and chemokines such as IL-8, IL-1β and chemokine ligand 1 and 3 by inhibiting endothelial proliferation [29, 30]. A variety of miRNAs take part in many stages throughout the process of atherosclerotic plaque development, and when dysregulated, such miRNAs cause destabilization and rupture of the plaque [28].

In vitro studies have identified several miRNAs assuming roles in angiogenesis and endothelial cell integrity. While miR-27b and miR-130 (whose relationship to CAD and atherosclerosis have been demonstrated) stimulate angiogenesis, miR-221 and miR-222 have been shown to inhibit it [28,29]. Moreover, miR-221 and miR-222 have been shown to control other miRNAs, particularly miR-223. It was reported that platelets of patients with atherosclerosis displayed increased miR-223, which participated in cholesterol biosynthesis, and was considered to be a potential mediator in the pathogenesis of atherosclerosis [31]. On the other hand, the angiogenesis-related miRNA-106b, -25, -92a and -21 levels were also demonstrated to increase in the atherosclerotic plaque [32]. The pro-atherosclerotic functions of miR-92a have been shown in many studies [32,33].

miR-143 and -145 have been shown to regulate the differentiation and functions of smooth muscle cells in the vessel wall structure. An in vitro study examining vascular diseases indicated that vessel wall stress may cause lower expression of these miRNAs [34,35]. It was demonstrated that these miRNAs may be necessary for neointima formation in atherosclerosis [36]. In the case of CAD, the expression of miR-126 may be reduced in response to blood flow due to atherosclerosis, triggering endothelial dysfunction [37].

• The relationship between ischemia/reperfusion injury and miRNA:

Studies have demonstrated the down regulation of miR-494 in the infarcted human heart and murine ischemia/reperfusion injury. In a myocardial infarction model that included transgenic mice with over-expression of miR-494 in the heart, the myocardial infarction size was reported to be significantly lower than the non-transgenic mice. Furthermore, in the case of adult cardiomyocytes, which were cultured similarly, short-term overexpression of miR-494 inhibited caspase-3 activity and lowered cell death in stimulated ischemia/reperfusion. The same study suggested that miR-494 targeted three different pro-apoptotic proteins and two different anti-apoptotic proteins along with the activation of Akt/PKB signaling pathway. Today, the Akt signaling pathway is known to have a cardio-protective effect against the injuries induced by ischemia/reperfusion [38].

Studies have shown that miRNAs get dysregulated during the response to the ischemic injury of the heart and actively contribute to cardiac remodeling after myocardial infarction [39]. A study conducted on pig heart tissue demonstrated increased presence of the miR-15 family in the infarcted areas of the heart as a response to ischemia/reperfusion. Mouse models displayed reduced infarction size and cardiac remodeling caused by therapeutic targeting of miR-15, and cardiac activity increased after myocardial infarction [39]. Other studies further demonstrated that miR-15a, miR-15b and miR-16 might reduce the size of infarct area and apoptosis, and protect the myocardium. Likewise, it has been reported that miR-15a and miR-15b expression increased whereas miR-16 expression showed no change in a murine ischemia/reperfusion model. In conclusion, down regulation of miR-15a and miR-15b was suggested to be a potentially significant strategy in preventing myocardial apoptosis induced by cardiac ischemia/reperfusion injury [40].

• The relationship between myocardial infarction and miRNA:

In a rat myocardial infarction model, insulin growth factor-1 (IGF-1) was shown to be targeted and down regulated by miR-1 and miR-206. Rat h9c2 myoblast cells, modified by miR-1, displayed significantly increased caspase-3 activity in comparison to the control group. Researchers have shown that miR-1 and miR-206 were associated with apoptotic cell death in myocardial infarction through the suppression of their target IGF-1 [41].

The immune system can get activated after myocardial infarction and trigger an acute inflammatory reaction. In a study examining altered expression of miR-146a, miR-150 and miR-155 in samples taken from autopsy materials of subjects with myocardial infarction with or without ventricular damage as well as normal heart tissue, it was reported that expression of the aforementioned three miRNAs was altered. miR-146a expression was increased in the cases with ventricular damage whereas miR-150 and miR-155 displayed reduced expression. The authors of the study also stressed upon an association between these micro RNAs and immunity acquired by birth [8].

A study conducted on patients with acute myocardial infarction found significantly increased miR-133 levels in the plasma in comparison to the control group. Similarly miR-328 was increased significantly in the plasma and whole blood of patients with acute myocardial infarction. In addition, a study comparing acute myocardial infarction patients accompanied by tachycardia or bradycardia with non-arrhythmic acute myocardial infarction patients determined no significant change in miR-133 and miR-328 in circulation [8].

Another study that examined the origin of miRNAs in circulation and their function in cardiovascular diseases determined that serum levels of miR-1 and miR-133a were significantly increased in patients with acute myocardial infarction, unstable angina pectoris and Takotsuba cardiomyopathy. The authors of the study suggested that miR-133a in the circulation of patients with cardiovascular diseases was driven by the damaged myocardium [42]. In a study that aimed to detect targeted miRNA in the plasma of patients with acute myocardial infarction, viral myocarditis, diastolic dysfunction and acute heart attack, miR-208b and miR-499, associated with cardiac myocytes in acute myocardial infarction patients, were shown to be significantly higher than the control group, however, no significant increase was detected in the case of patients with viral myocarditis. Only miR-499 displayed an increase in patients with

acute heart attack whereas the miRNAs did not present any significant change in patients with diastolic dysfunction [43].
In another study that aimed to identify cardiac-specific miRNA in the circulation during acute myocardial infarction, the candidates, namely miR-1, miR-133a and miR-499 levels were found to be much lower than those displayed by the plasma of healthy individuals, moreover, miR-208a was reported to be absent [8]. However, in the case of rats with acute myocardial infarction, the plasma levels of these miRNAs were demonstrated to have increased to a significant extent. Particularly miR-208a could not be detected in the plasma within one hour after coronary artery occlusion, although it then increased significantly. As a result, these four miRNAs were reported to be significantly higher in patients with acute myocardial infarction in comparison to healthy individuals, those without coronary diseases and patients with other cardiovascular diseases [28].
In a study investigating the role of miRNAs in the early phase of acute myocardial infarction, the miRNA expression profile in the rat heart was compared to non-infarction areas six hours after acute myocardial infarction. The results indicate that the expression of miR-21 was down regulated significantly in the infarction areas while it was up regulated in the border areas (infarction and non-infarction). When the miR-21 level was through an adenoviral construct in cultured cardiac myocytes, the myocardial infarction area was demonstrated to be reduced by 29% in 24 hours. This study further highlighted that miR-21 had a protective effect in ischemia-induced cellular apoptosis by targeting programmed cell death 4 and activator protein 1 [44]. In another study conducted on a population of patients with acute myocardial infarction and healthy individuals (control group), among the former, miR-30a was reported to be highly expressed at 4, 8 and 12 hours, which was the same for miR-195 at 8 and 12 hours, after the beginning of acute myocardial infarction. On the other hand, within the same group, miR-let-7b was expressed at a lower level at the beginning of the infarction. However, all three miRNAs peaked at 8 hours in patients with acute myocardial infarction [10].
After myocardial infarction, miR-24 has been reported to play a key role in the development of cardiac fibrosis. Studies have demonstrated a positive correlation between miR-24 and fibrosis in hypertrophied hearts. miR-24 mediated regulation was demonstrated to be reduced in myocardial infarction, and miR-24 expression was reported to be closely associated with the structuring of extracellular matrix. In vivo studies further reported that, with intramyocardial injection of a lentiviral miR-24 construct, miR-24 was accumulated in the borders of infarct areas of the heart two weeks after myocardial infarction and resulted in increased cardiac functions and reduced cardiac fibrosis. An in vitro study demonstrated that miR-24 may reduce fibrosis by reducing the migration and differentiation of cardiac fibroblasts, and further reported that miR-24 plays a critical role in the function of cardiac fibrosis and cardiac fibroblasts after myocardial infarction as well as the furin-TGFB signaling pathway by targeting furin, a protease related to the TGFB activation process [45].

• The relationship between atherosclerosis and miRNA:
Th1 cells that are important components of the acquired immune system, are characterized by the generation of interferon-gamma (IFN-ϑ) that activates the macrophages and leads to "cell-mediated immunity". Th1 cells are up regulated in the development of atherosclerosis. Furthermore, miR-146a is highly expressed in autoimmune diseases induced by Th1. A study conducted by Guo et al. demonstrated the increased expression of miR-146a in peripheral blood mononuclear cells of patients with acute coronary syndrome. An in vitro study reported that miR-146a induced the expression of the p65 subunit of the inflammatory transcription factor NF-kappaB along with MCP-1 and TNF-α, which are pro-inflammatory cytokines that play a key role in patients with atherosclerosis [46].
miRNAs may assume a significant role in the pathogenesis of atherosclerosis in the context of endothelial vascular smooth muscle cells and cardiovascular homeostasis. With the knockdown of miR-10a in cultured human aortic endothelial cells, the expressions of vascular cell adhesion molecule 1 (VCAM-1) and endothelial-leukocyte adhesion molecule 1 (E-selectin), which play significant roles in the accumulation of leukocytes in the inflammation area, were inhibited [47]. Another study comparing arteries with atherosclerosis obliterans and normal control arteries in terms miRNA expression revealed that miR-21 was particularly localized in the arterial smooth muscle cells. Additionally, cell proliferation and migration were reduced to a significant extent in cultured arterial smooth muscle cells with the inhibition miR-21, and tropomyosin was determined to be the target of miR-21 [48]. Another study found miR-126 necessary for endothelial recovery and reported that lesion formation was stimulated by the down regulation of miR-145, playing a role in the differentiation of smooth muscle cells [49].
Macrophages in the atherosclerotic plaques execute inflammatory responses by the phagocytosis of lipoproteins and dead cells [50]. miRNAs control the activity and differentiation of macrophages through various signaling pathways [51]. Nazari et al. reported that miR-155 was specifically expressed in atherosclerotic plaques and pro-inflammatory macrophages. Mice lacking miR-155 displayed a reduced number of lesional macrophages and reduced plaque size after partial carotid ligation. In addition, the same study demonstrated the increased expression of BCL-6, a transcription factor that reduced miR-155 mediated pro-inflammatory NF-κB signal transduction. It was further reported that reduced expression of BCL-6 in the macrophages of mice where miR-155 was knocked-down increased plaque formation and expression of the CCL2 chemokine [52].
In conclusion, a number of miRNA-based strategies are being actively explored in many labs around the world for the treatment of cardiovascular diseases and to ensure their clinical implementation in the near future. Further studies revealing the disease pathways related to miRNAs will enable new methods for the treatment of diseases and pave the way for different treatment approaches.

Competing interests
The authors declare that they have no competing interests.

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